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Synthetic biology with artificially expanded genetic information systems. From personalized medicine to extraterrestrial life

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ABSTRACT

Over 15 years ago, the Benner group noticed that the DNA alphabet need not be limited to the four standard nucleotides known in natural DNA (1,2). Rather, twelve nucleobases forming six base pairs joined by mutually exclusive hydrogen bonding patterns are possible within the geometry of the Watson-Crick pair (Fig. 1). Synthesis and studies on these compounds have brought us to the threshold of a synthetic biology, an artificial chemical system that does basic processes needed for life (in particular, Darwinian evolution), but with unnatural chemical structures. At the same time, the artificial genetic information systems (AEGIS) that we have developed have been used in FDA-approved commercial tests for managing HIV and hepatitis C infections in individual patients, and in a tool that seeks the virus for severe acute respiratory syndrome (SARS). AEGIS also supports the next generation of robotic probes to search for genetic molecules on Mars, Europa, and elsewhere where NASA probes will travel.

INTRODUCTION

In natural DNA, two complementary strands are joined by a sequence of Watson-Crick nucleobase pairs. These pairs obey two rules of complementarity: size complementarity (large purines pair with small pyrimidines) and hydrogen bonding complementarity (hydrogen bond donors pair with hydrogen bond acceptors). The former is necessary to permit the aperiodic crystal structure that underlies faithful replication of genetic information essential for life. The latter achieves the specificity that gives rise to the simple rules for base pairing ("A pairs with T, G pairs with C") that underlie genetics and molecular biology.

The elegance of the structure of terrean DNA prompts the general question: Is its structure universal? If life arose in the cosmos independently of life on Earth, would it use exactly the same genetic material? Or can alternative chemical structures support rule-based molecular recognition as well? To answer these questions requires that alternative structures be imagined, and that the power of contemporary synthetic organic chemistry be applied to prepare them in the laboratory. There, they can be studied to determine their suitability as genetic systems.

Ultimately, we hope to develop a "synthetic biology". The idea of synthetic biology is embedded deep within the tradition of organic chemistry. Over two centuries, chemistry developed through the isolation and structural characterization of natural products. As their deconstructive powers grew, chemists targeted larger biomolecules, first proteins and nucleic acids, and then supramolecular structures such as the nucleosome and the ribosome.

Chemists then complemented deconstruction with attempts to synthesize new structures that were *not* identical to natural products, but reproduced behaviors that natural biomolecules display. Biomimetic Chemistry, as it came to be called, had modest goals at first, for example, to create a molecule that catalyzes a chemical transformation catalyzed by a natural enzyme. The next step in the development of this tradition is to reproduce advanced, complex, and dynamic behaviors of biological systems, including genetics, inheritance, and evolution. These goals define synthetic biology as a field.

To exploit AEGIS as a component of a synthetic biology, several physical organic features of its constituents must be addressed. First, we are interested in obtaining heterocyclic systems implementing certain hydrogen bonding patterns that do not epimerize (Fig. 2) (3). Further, we wish to enhance the interaction between polymerases and AEGIS components. This will allow terrean polymerases to copy DNA analogs containing AEGIS components, and then copy the copies. This two step process is a key to replication in these systems.

RESULTS AND DISCUSSION

Enhancing the incorporation of AEGIS components by polymerases required altering the polymerase itself via point mutation. Here, two amino acids were replaced in HIV reverse transcriptase (Y188L E478Q) to create a variant that incorporated the pyDAD-puADA component with high fidelity (Fig. 2).

Managing epimerization in the pyDDA system requires a structure that diverts the electron flow. To this end, we designed a derivative carrying the nitro group (Fig. 3).

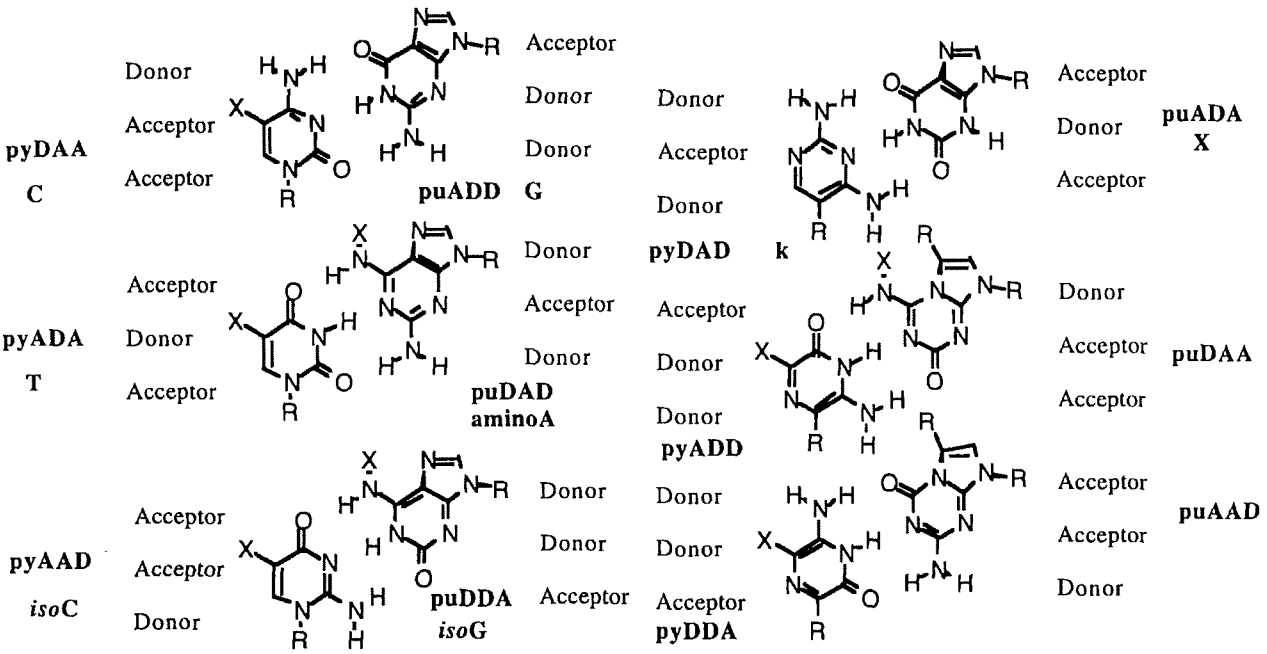

Fig.1 Twelve nucleobase analogs joined in six mutually exclusive hydrogen bonding patterns are possible within the Watson-Crick in line geometry.

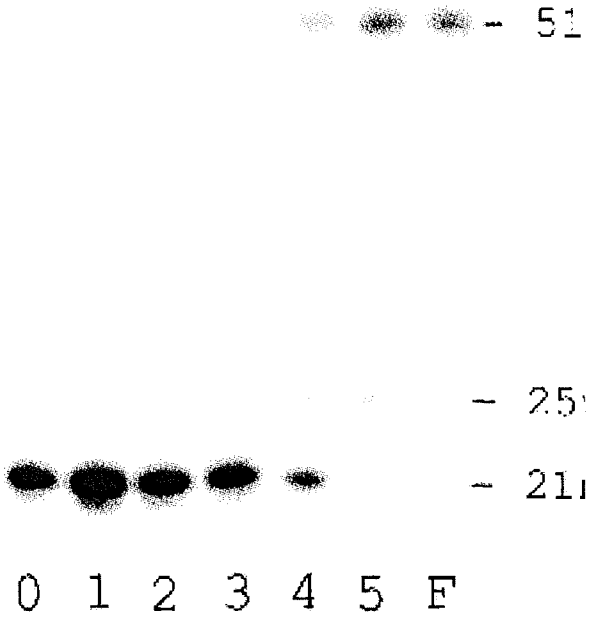

Fig. 2 Polymerase chain reaction showing five rounds of PCR amplification of a six letter genetic information system, including A, T, G, C, pyDAD, and puADA. F is full length product. Numbers indicated rounds of selection. An engineered HIV reverse transcriptase was used

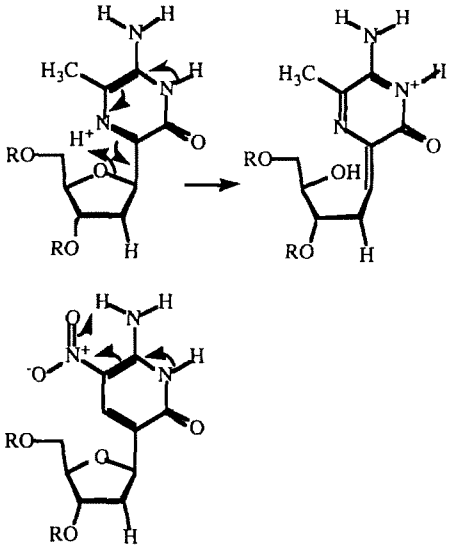

Fig. 3 Epimerization in the pyDDA nucleobase should be suppressed by a nitro substituent.

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